

Fish Health Indicator Scores and Grades for the 2025 Gladstone Harbour Report Card: ISPO23-2025

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VERSION HISTORY

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1.1	Initial draft of interim report – to GHHP	NF, AI, AA, AC, GC	31/07/2025
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EXECUTIVE SUMMARY

Fish are widely recognised as effective ecological indicators of environmental condition due to their sensitivity to a wide array of stressors, varying range of trophic representation, and long lifespans. This makes them suitable indicators to assess acute and chronic ecological change. In 2018, CQUniversity partnered with the Gladstone Healthy Harbour Partnership (GHHP) and the Fisheries Research and Development Corporation (FRDC) to develop and implement fish health indicators for inclusion in the Gladstone Harbour Report Card, using a modified version of the Health Assessment Index (HAI). This assesses the cumulative impact of environmental stress on individual fish, with a high score indicating greater physiological stress and poorer health. The Fish Health Indicator monitoring program can provide early warning signs of ecosystem degradation, especially when physiological, morphological, and parasitological traits are monitored, and can thus capture sublethal effects. In 2024, GHHP commissioned CQUniversity to re-establish the Fish Health Indicator monitoring program after a three-year monitoring break to generate grades for inclusion in the 2025 Gladstone Harbour Report Card.

Fish were sampled from the 'northern', 'central', and 'southern' regions of Gladstone Harbour in February-March 2025 (wet season), and in May-June 2025 (dry season) to achieve an even spread of fish catch and effort between locations and allow comparisons between seasons. Fish taxa were targeted that exhibit smaller home ranges, such fish are more likely to show health-related impacts specific to that part of the harbour. These included bream (pikey bream *Acanthopagrus pacificus* and yellowfin bream *Acanthopagrus australis*) and barred javelin (*Pomadasys kaakan*), in addition to smaller catches of blue catfish (*Neoarius graffei*) and sea mullet (*Mugil cephalus*). Fish were caught on baited-hooks (except sea mullet, which are schooling detritivores so were caught with nets) with 5 – 7 fish (>25 cm length) per region per sampling season. All fish were photographed, measured (total length, standard length, and fork length) and weighed, and both internal and external tissues and organs were examined for abnormalities, parasites, lesions or erosion. A total HAI score for each individual fish was calculated (sum of all organ scores) and then averaged for each fish taxon. All scores were reported as individual species, except "bream" (pikey and yellowfin), which were pooled due to their similar ecological characteristics.

A total of 353 fish were caught across the 10-day sampling period from January to June 2025, comprising 20 species, with 89 fish retained for laboratory analysis. The majority of fish presented good external health with clear eyes and healthy gills, with only a small number with minor skin irritation (n=9) and fin damage (n=6). Fish HAI scores were relatively low across both sampling seasons (wet vs dry) and within each harbour sampling zone (north vs central vs south), with the highest average HAI of 40 recorded for bream from the southern zone during the wet season. Spatio-temporal differences were not significant in HAI for either bream or barred javelin, and both scored relatively well (19.29 bream, 13.9 barred javelin) indicating good overall health. No statistical analysis could be carried out on blue catfish and mullet due to lack of replication, but the HAI was also low/good (17.14 blue catfish, 10.91 for mullet). Using the GHHP grading scale, an overall harbour score and grade was determined by averaging the scores across the five species groups.

This resulted in a Grade A for barred javelin (score of 0.94), blue catfish (0.98), sea mullet (0.98) and bream (0.85) with an overall grade A for the Harbour (0.91).

Whilst the laboratory and data analysis have remained unchanged since 2018/19, the sampling effort has varied (seasonality, fishing method, zonation of harbour, targeted species) between years. This influences both the species and size ranges of fish caught for analysis, and the ability to undertake trend analysis. In 2025 the field sampling method changed from gillnetting to primarily to baited hook and line fishing, to concentrate fishing effort. Control sites were not funded for monitoring in 2025, but it would be beneficial for GHHP to consider sampling at two control sites during intensive assessment years if budget allows, to avoid misinterpreting more widespread changes as localised impacts. The benchmark of a score of 10 (minor damage to one organ/metric) and worst-case scenario (WCS) score of 70 remain suitable for retention for calculating the HAI score and grade. In future, preserved tissue samples collected in 2025 could provide valuable information for identifying environmental impacts, if alternative funding sources could be procured for histopathology or bioaccumulation analyses.

INTRODUCTION

Fish are widely recognised as effective ecological indicators of environmental condition. Their sensitivity to a broad range of stressors, broad trophic representation, and relatively long lifespans make them suitable for assessing both acute and chronic ecological change (Sajina et al., 2021; Yancheva et al., 2020). Because they are relatively mobile yet still reflect cumulative environmental exposures, fish can provide a more integrated view of ecosystem health than many physicochemical measures alone (Schlacher, Mondon, and Connolly, 2007). Their socio-economic importance also means fish-based indicators tend to resonate well with the community and decision-makers, making them useful for environmental reporting frameworks such as report cards.

In 2018, CQUniversity partnered with the Gladstone Healthy Harbour Partnership (GHHP) and the Fisheries Research and Development Corporation (FRDC) to develop and implement fish health indicators for inclusion in the Gladstone Harbour Report Card. A modified version of the Health Assessment Index (HAI) was selected for use from 2019 onwards (Flint et al., 2018). Originally developed by Adams et al. (1993), the HAI integrates observations of parasite load and organ condition (including liver, kidney, gills, skin, spleen, and hindgut) to assess the cumulative impact of environmental stress on individual fish. Higher HAI scores indicate greater physiological stress and poorer health. Between 2019 and 2021, these assessments were incorporated into the annual Gladstone Harbour Report Cards (Flint et al., 2019a, 2020, 2021a), forming part of a broader ecosystem health evaluation for the region. The HAI method offers several practical advantages in applied monitoring contexts. It allows for integration of multiple types of visible indicators without requiring laboratory-based biochemical or histological analysis, which can be time-consuming, costly, or logistically impractical in large-scale field programs. It is also species-flexible and can be applied across commonly caught species in estuarine and coastal waters, allowing for variable sampling regimes.

While many Australian report cards emphasise population and community-level indicators such as species composition or probability of encounter (e.g., FPRH, 2024; Healthy Rivers to Reef Partnership, 2025; Healthy Land & Water, 2023), these approaches have limitations. They often require extensive sampling effort to capture temporal and spatial variability and may struggle to detect sublethal stress or early-stage impacts. Individual-level health indicators, in contrast, can provide early warning signs of ecosystem degradation, especially when physiological, morphological, and parasitological traits are monitored alongside traditional population metrics (Whitfield and Elliott, 2002; Almeida et al., 2023). The HAI strikes a useful balance by capturing sublethal effects in individual fish.

Recent research supports the use of various fish traits as sensitive indicators of environmental condition. Morphological changes in tissues such as gills, liver, and hepatopancreas have been linked to exposure to pollutants, including pesticides and heavy metals (da Silva Montes et al., 2020; Yancheva et al., 2020). Histopathological lesions, particularly in the liver and kidney, are associated with chronic contaminant exposure and may indicate irreversible damage (Assis de Brito Carvalho et al., 2022). However, histological approaches are more resource-intensive than the HAI, which provides a practical, cost-effective field-based method for identifying early-stage tissue damage or visible abnormalities that can serve as reliable proxies for stress.

Fish skin has also emerged as a promising biomonitoring tissue, with epidermal thickness and mucous cell patterns showing strong correlation with ecological quality (Orso et al., 2024). Although these responses are biologically meaningful, sampling and processing of skin tissue for histology presents similar logistical barriers to those associated with liver and gill analysis. These methods may have value in more targeted or research-oriented contexts but are less suited to routine monitoring frameworks like the Gladstone Harbour Report Card, except in cases where externally visible pathologies are identified or an impact is suspected.

Parasitological indicators also offer promise. Ectoparasite and endoparasite loads have been shown to vary with water quality, season, and host condition and may serve as indirect measures of contaminant exposure or ecosystem imbalance (Biswas et al., 2023; Keke et al., 2020). Some helminths, for instance, bioaccumulate pollutants at higher concentrations than their fish hosts, making them useful sentinels for heavy metal contamination. However, recent work has highlighted that parasite load and diversity may respond differently depending on habitat structure and stressor type, with increased load in degraded areas but reduced diversity in structurally simple or highly disturbed environments (Almeida et al., 2023). In addition, the biology and even the taxonomy of Australian marine parasites remain poorly understood. The HAI method incorporates overall parasite counts as part of a broader health assessment, allowing this information to be considered alongside other organ-level indicators rather than in isolation.

While fish-based monitoring typically focuses on a single species or a small number of target species, emerging work has called attention to the potential value of multispecies biomonitoring (Cateau et al., 2021). This approach improves confidence by capturing species-specific variation in sensitivity, exposure, and response to contaminants. Integrated biomarker frameworks such as the Integrated Biomarker Response (IBR) index can help synthesise results across multiple tissues, biomarkers, and species, though they have not yet been applied in the Gladstone context. The HAI method remains feasible for application across multiple species and sites, providing consistent outputs that are interpretable within existing report card frameworks.

Following a three-year monitoring break and a review of monitoring design to align with funding availability, in 2024 GHHP commissioned CQUniversity to re-establish the Fish Health Indicator monitoring program, beginning with a detailed assessment

in 2024-25. This more intensive sampling is intended to be repeated every third year, with lighter monitoring occurring in intervening years.

The objective of this project is to assess fish health in Gladstone Harbour using the established indicator framework (Flint et al., 2019a,b) and generate grades and scores for inclusion in the 2025 Gladstone Harbour Report Card. This work builds on CQUniversity's previous research on fish indicators in the region (Flint et al. 2019a,b, 2020, 2021a). The HAI method remains central to this approach, offering a field-ready, species-flexible, and ecologically meaningful way to track fish health through time and between sites, and inform regional reporting.

METHODS

Permits and approvals

The following permits and approvals are in place for this research:

- General Fisheries Permit (Queensland Department of Agriculture and Fisheries; Permit Number 272574)
- Animal Ethics Approval (CQUniversity Animal Ethics Committee; Approval Number 25315)
- Authorisation for research in the Great Barrier Reef Marine Park (Approval Number G18/03-029)
- Field Work Risk Assessment (CQUniversity OHS Unit)

Sampling design

Fish were sampled from the 'northern', 'central, and 'southern' regions of Gladstone Harbour (Figure 1) in February-March 2025 (wet season sample), and again in May-June 2025 (dry season sample). The sampling strategy in Gladstone Harbour was developed during previous years, to achieve an approximately even spread of fish catch and effort between the northern, central and southern areas of the harbour, while facilitating comparisons between wet and dry season conditions.

The primary aim of sampling was to collect target fish taxa considered reliable to catch and known to exhibit smaller home ranges, thus more likely to show health-related impacts specific to that part of the harbour. Discussions with the GHHP Independent Science Panel, based upon past research efforts in the region (e.g. Flint et al 2018, 2021) identified bream (pikey bream *Acanthopagrus pacificus* and yellowfin bream *Acanthopagrus australis*) and barred javelin (*Pomadasys kaakan*) as key targets. Blue catfish (*Neoarius graffei*) were also sampled if captured incidentally, and sea mullet (*Mugil cephalus*) were also targeted on one sampling day during the wet season to acquire data for this detritivore that often moves upstream into inlets and tributaries, providing additional ecological context. Understanding the mobility of fish in Gladstone Harbour is an important consideration that was considered when confirming target species (for more details see Flint et al., 2019).



Figure 1. Location of the Gladstone Harbour Zones. Inset, environmental grading system used in the annual report card. Source: GHHP, 2022.

Field sampling methods

Fish were collected from the field using baited hooks on braided fishing line. Multiple rods were set during each sampling period, with bait including dead prawns, squid, and mullet purchased from local retailers. Typically, a fishing ground was identified using a depth sounder and side-scan sonar, with fishing times often targeted to the two-to-three hours either side of the high tide. Baited hooks were set for approx. 20 – 30 minutes at each site and if no target species were caught during that time, the next site was fished.

Approximately 5 – 7 individual fish of each target species were sought per region in each sampling season ("wet" from Jan-Mar; "dry" from May-Jun). Target specimens measuring 25cm or longer were preferred, although some smaller individuals were retained to provide enough sample material for meaningful comparisons. Bycatch (including under-sized target species) was

recorded, noting species and also total length and weight when time permitted. All bycatch was de-hooked and returned to the capture location. Cartilaginous fishes (sharks and rays) were rarely encountered but were also recorded as bycatch.

On the final day of the wet season sampling, sea mullet were initially targeted using a 50m-long gill net with a stretched mesh size of 4.5", but this did not result in any target species captured at any of four sites. Subsequently, a 3-m cast net with 1" mesh size was used to target schools of mullet in shallow waters as the tide receded. Fish that were retained for further analysis were assigned a unique identification code (site abbreviation and fish number) and either processed immediately or placed into an aerated swim tank to be kept alive until on-board processing. Prior to processing, fish were euthanised by swimming them in an aerated solution of the fish anaesthetic MS-222 in seawater for a minimum of 15 minutes, to overdose and humanely kill them. Once dead, each fish was photographed, measured (total length, standard length, and fork length) and weighed, and the skin, fins, gills and eyes were examined for abnormalities, parasites, lesions or erosion. Additionally, a gill arch sample was collected from each fish and fixed in 10% formalin. Field collection of other tissues wasn't possible, however, as opening the visceral cavity in the field would confound the comprehensive assessment of internal organ condition once fish were returned to the laboratory. All retained fish were individually bagged with their unique identifier tag and placed in an ice slurry for return to the laboratory as soon as possible on the same day.

Laboratory methods

Retained fish from all sites were returned to the lab at the CQUniversity, North Rockhampton Campus for same day mid-level pathological examination as described by Cowled (2016). Pathological examination also included the dissection of additional organs (liver, kidney, gonad, spleen, heart, muscle/skin) and fixation in 10% formalin for later histopathological analysis, if required and funded.

Each fish was dissected by a team of two researchers, using the step-by-step protocol detailed in Flint et al. (2019). Fins, spleen, hindgut, kidney, skin, liver, eyes, gills and parasites were scored following the protocol originally developed by Adams et al. (1993) and modified by Wesche et al. (2013) (Table 1). Notes on the condition of other tissues and organs (abdomen, heart, mesentery fat, bile) were also taken for future reference and interpretation.

Calculating fish condition measures

Scores for the organs of each fish were recorded based on data collected during fish dissections. Total HAI score for each individual fish was calculated (sum of all organ scores) and then the average of the total HAI scores was calculated for each fish taxon. These total scores at taxa level were averaged to give the overall harbour score. Blue catfish, barred javelin and sea mullet were reported as individual species. The species "bream" includes two species (pikey and yellowfin), which were pooled due to their similar ecological characteristics and to allow for higher sample sizes.

Statistical analytical methods

Spatio-temporal differences of HAI scores were statistically tested using Permutational Multivariate Analysis of Variance (PERMANOVA (Anderson 2017)). Akin to traditional ANOVA analysis, PERMANOVA confers distinct advantages for field and environmental data sets that frequently violate the parameter assumptions of ANOVA. Principally, PERMANOVA completes a robust analysis even with un-balanced data sets (replicates and/or treatment levels), and it's permutational approach to calculating *P*-values frees it from the traditional constraints of requiring normally distributed data and homogenous variances. While chiefly designed for the analysis of multivariate data sets, using PERMANOVA to analyse univariate data replicates the results of traditional ANOVA calculations with excellent fidelity.

For this research, the HAI scores calculated for bream and barred javelin were compared across sampling seasons (wet vs dry) and zones (north vs central vs south). Seasons were treated as 'fixed' and zones treated as 'fixed and orthogonal' to seasons. The PERMANOVA analyses were based on a Euclidean resemblance matrix, with up to 9,999 permutations of the data to increase the robustness of the analysis. A statistical analysis of blue catfish HAI could not be completed due to a lack of adequate replication across the data set. Similarly, a statistical analysis of mullet HAI could not be completed due to fish only being collected from one zone in the dry season.

Table 1. Variables and substituted values used in the Health Assessment Index for this project (source: Wesche et al., 2013).

Variable	Variable condition	Field Designation	Substituted Value
Fins	No active erosion	F0	0
	Light active erosion	F1	10
	Severe active erosion	F2	20
Spleen	Normal: black, very dark red or red	B	0
	Normal: granular, rough appearance	G	0
	Nodular, containing fistulas or nodules	D	30
	Enlarged	E	30
	Other: aberration not fitting any above	OT	30
Hindgut	Normal, no inflammation or reddening	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal: firm, dark, flat	N	0
	Swollen: enlarged or swollen	S	30
	Mottled: gray discolouration	M	30
	Granular in appearance and texture	G	30
	Urolithiasis or nephrocalcinosis	U	30
	Other: aberration not fitting any above	OT	30
Skin	Normal: no aberration	SK0	0
	Mild skin aberrations	SK1	10
	Moderate skin aberrations	SK2	20
	Severe skin aberrations	SK3	30
	Extensive redness as a rash. Scales intact	SK4	40
Liver	Normal: solid red or light red color	A,B	0
	'Fatty' liver, 'coffee with cream' colour	C	30
	Nodules or cysts in liver	D	30
	Focal discolouration	E	30
	General discolouration	F	30
	Other: deviation not fitting any above	OT	30
Eyes	No aberration, good, clear eyes	E0	0
	Fresh haemorrhage (eg net damage)	EOa	0
	Opaque eye (one or both)	E1	30
	Cloudy and swollen, red or haemorrhaging	E2	30
	Ruptured (one or both)	E3	30
Gills	Normal: no apparent aberrations	N	0
	Frayed, ragged appearance	F	30
	Clubbed, swelling of tips	C	30
	Marginate: light discoloured margin	M	30
	Pale, very light colour	P	30
	Other	OT	30
Parasites	No observed parasites	P0	0
	Few observed parasites	P1	10
	Moderate parasite infestation	P2	20
	Numerous parasites	P3	30

RESULTS

Fish catch

A total of 353 fish were caught across the 10-day sampling period from January to June 2025, comprising 20 species (Table 2). Of these captures, 89 fish were retained for laboratory analysis (Table 3).

A reasonably consistent abundance of barred javelin and bream (yellowfin and pikey bream) were retained across each sampling region, except for the Northern zone during the “dry season” (May-June) sampling where no grunter of appropriate size could be captured. Blue catfish were caught in relatively low numbers, with specimens only caught and retained in the wet season. When sea mullet were targeted on the final day of the dry season sampling, 11 specimens were retained for laboratory analysis.

Field processing of retained fish noted very few external health-related issues across the sampling effort. Some slight skin irritation (redness) around the throat & pelvic fins was observed for nine fish, with some minor fin damage observed for six fish, which may be indicative of prior injuries. Overall, the majority of fish presented good external health, with clear eyes and healthy gills.

Bycatch included small specimens of the target species, as well as a mix of common coastal and estuarine species for Central Queensland. Moses perch (*Lutjanus russellii*) and estuary cod (*Epinephelus coioides*) were the two most common by-catch species, numbering 133 and 44 individuals, respectively, across the sampling work.

Table 2. Fish species (listed by common name) and abundance, by capture region. Includes all retained target species and bycatch.

Sampling period	Fish species	GHHP region		
		North	Central	South
Wet season (Feb-Mar 2025)	Barred javelin (<i>Pomadasys kaakan</i>)	5	7	7
	Spotted grunter (<i>Pomadasys commersonii</i>)			1
	Yellowfin bream (<i>Acanthopagrus australis</i>)	7	7	4
	Pikey bream (<i>Acanthopagrus pacificus</i>)	1		
	Blue catfish (<i>Neoarius graeffei</i>)	5	2	
	Sea mullet (<i>Mugil cephalis</i>)			
	Moses perch (<i>Lutjanus russellii</i>)	10	3	43
	Estuary cod (<i>Epinephelus coioides</i>)	2	4	4
	Grass sweetlip (<i>Lethrinus laticaudis</i>)	1		5
	Giant trevally (<i>Caranx ignobilis</i>)			1
	Bull shark (<i>Carcharinus leucas</i>)	2		
Dry season (May-Jun 2025)	Barred javelin (<i>Pomadasys kaakan</i>)	8	9	8

	Spotted grunter (<i>Pomadasys commersonnii</i>)			4
	Yellowfin bream (<i>Acanthopagrus australis</i>)	21	7	5
	Pikey bream (<i>Acanthopagrus pacificus</i>)	13	3	2
	Blue catfish (<i>Neoarius graeffei</i>)			
	Sea mullet (<i>Mugil cephalis</i>)	11		
	Moses perch (<i>Lutjanus russellii</i>)	60	10	7
	Estuary cod (<i>Epinephelus coiodes</i>)	23	9	2
	Grass sweetlip (<i>Lethrinus laticaudis</i>)	12		1
	Mangrove jack (<i>Lutjanus argentimaculatus</i>)	1		
	Silver javelin (<i>Pomadasys argenteus</i>)	4		
	Barracuda (<i>Sphyræna barracuda</i>)	2		
	Sand whiting (<i>Sillago ciliata</i>)	3		
	Grinner (<i>Trachinocephalus myops?</i>)	1		
	Sickle fish (<i>Drepane punctata</i>)		1	1
	Butter bream (<i>Monodactylus argenteus</i>)			1
	Fringefin trevally (<i>Pantolabus radiatus</i>)			2
	Bartail flathead (<i>Platycephalus indicus</i>)			1

Table 3. Number of target fish species retained for laboratory analysis for each region and sampling period (wet vs dry season).

Sampling period	Fish species	GHHP region		
		North	Central	South
Wet season (Feb-Mar 2025)	Barred javelin (<i>Pomadasys kaakan</i>)	5	7	7
	Yellowfin bream (<i>Acanthopagrus australis</i>)	7	7	4
	Pikey bream (<i>Acanthopagrus pacificus</i>)	1		
	Blue catfish (<i>Neoarius graeffei</i>)	5	2	

Dry season (May-Jun 2025)	Barred javelin (<i>Pomadasys kaakan</i>)		6	4
	Yellowfin bream (<i>Acanthopagrus australis</i>)	11	5	4
	Pikey bream (<i>Acanthopagrus pacificus</i>)		1	2
	Blue catfish (<i>Neoarius graeffei</i>)			
	Sea mullet (<i>Mugil cephalis</i>)	11		

Fish condition by species and region

Fish HAI scores were relatively low across both sampling seasons (wet vs dry) and within each harbour sampling zone (north vs central vs south). The highest average HAI recorded was 40, for bream sampled in the southern zone during the wet season. Statistically comparing fish HAI scores across seasons and sampling zones revealed no significant spatio-temporal difference in HAI either for bream or for barred javelin ($P > 0.05$; Appendix 1).

Overall, bream scored higher average HAI values than barred javelin (Figure 2), though in the context of the highest (worst condition) HAI value of 270, both species scored well (19.29 bream and 13.9 barred javelin) and indicated good overall health. While some spatio-temporal patterns may be suggested by the data (e.g. decline of bream HAI in the southern zone from wet to dry seasons: Figure 1), the variation among the data obscured such patterns during the statistical analysis, with additional replicates likely to rectify such outcomes in future sampling events.

The HAI scores for blue catfish and for mullet could not be statistically analysed due to either a lack of replication (blue catfish) or a lack of sampling across seasons and zones (mullet). Even so, the average HAI for both species was also relatively low, scoring 17.14 for blue catfish and 10.91 for mullet (Table 4).

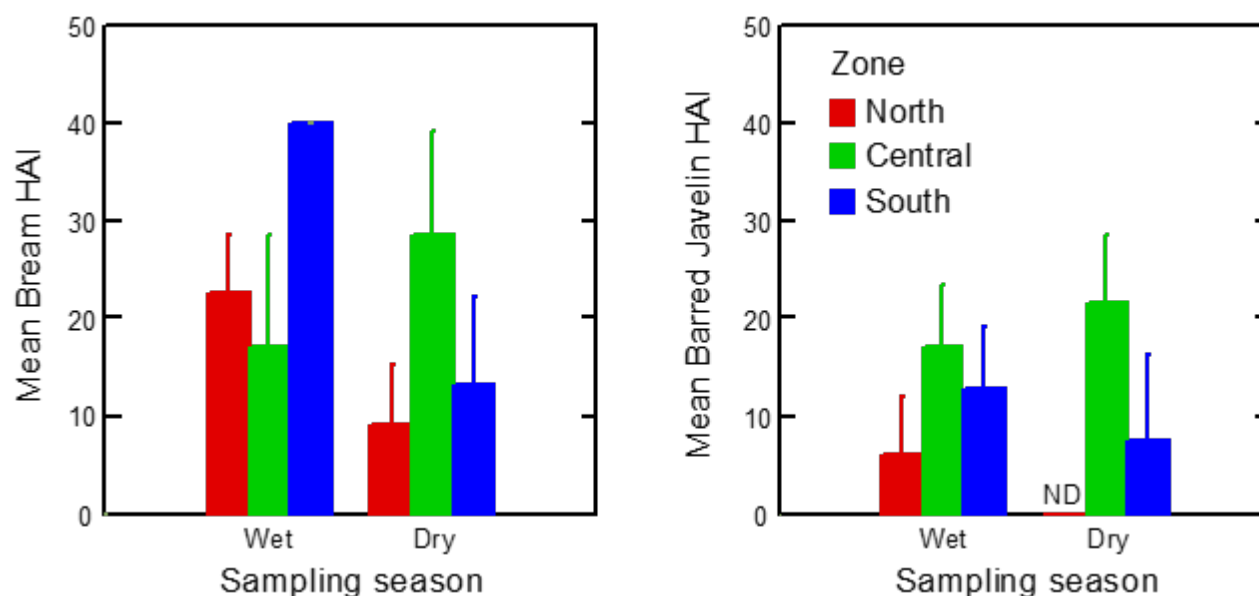


Figure 2. Comparison of mean HAI scores for bream and barred javelin across sampling seasons (wet vs dry) and zones (north vs central vs south). Note that barred javelin were not caught in the northern zone during the dry season sampling; represented as 'ND' in the figure.

FISH HEALTH INDICATOR RESULTS FOR GLADSTONE HARBOUR IN 2025

In 2018, HAI was identified as the most appropriate fish health indicator for immediate implementation in the Gladstone Harbour Report Card (Flint et al., 2018; 2019; 2020; 2021). The metric requires gross pathological analysis during a detailed laboratory dissection and produces a composite metric that integrates evaluations of the condition of multiple organs and tissues. The premise of the index is that scores will cumulatively reflect the acute and chronic stressors present in the fish's environment, with poorer anatomical condition resulting in higher HAI scores and thus indicative of a more stressful environment (Adams et al., 1993). The modified version of the HAI used in this study was also used by Wesche et al. (2013) during the fish health investigation in Gladstone Harbour in 2011-2012.

Measures and baselines

In 2025, HAI was again calculated for each of the 89 fish within the four target taxa by scoring and summing gross pathology scores for the following measures: skin, eyes, fins, gills, spleen, kidney, hindgut, liver, and parasite load. The best possible score for each measure, and in total, is 0. Any increase from a score of 0 indicates the identification of gross pathologies visible during a routine necropsy dissection. The highest (worst) score for each individual measure is 30 and in total is 270.

The HAI is designed to be used as a summed average for a sample population (Adams et al., 1993). Using this method, the Gladstone Harbour-wide HAI results (over nine measures) were determined, by taxon (Table 4). Average HAI ranged from 11 (sea mullet) to 19 (bream).

Table 4. Average measure and HAI total scores, calculated for fish caught in Gladstone Harbour from January to June 2025. Individual scores for each organ range from 0-30. Total individual HAI scores range from 0-270. The category "Bream" includes pikey bream and yellowfin bream.

Taxa / Measure	Barred javelin (n = 29)	Blue catfish (n = 7)	Sea mullet (n = 11)	Bream (n = 42)
Skin	0.00	0.00	0.00	0.24
Eyes	0.00	4.29	0.00	0.00
Fins	0.00	0.00	0.00	0.00
Gills	0.00	4.29	0.00	1.43
Spleen	0.00	0.00	0.00	0.00
Kidney	2.07	4.29	2.73	8.57
Hindgut	0.00	0.00	0.00	0.00
Liver	11.79	0.00	5.45	5.00
Parasites	0.34	4.29	2.73	4.05
HAI score	13.79	17.14	10.91	19.29

Scoring the HAI

Using the previously determined Benchmark (B) of 10 and Worst Case Scenario (WCS) of 70, standardised HAI scores and grades were calculated using a distance from the benchmark method (Flint et al., 2019). The standardised scores and grades calculated using collected in 2025 are provided in Table 5.

The distance from the benchmark function used is as follows:

$$\text{Calculated score} = 1 - ((x-B)/(WCS-B))$$

Where: x = average HAI; B = benchmark; WCS = worst case scenario

Table 5. Calculation of standardised HAI scores for Gladstone Harbour using data collected in 2025.

Species	Average HAI	Benchmark	WCS	Calculated score
Barred Javelin	13.79	10	70	0.94
Blue Catfish	17.14	10	70	0.88
Sea Mullet	10.91	10	70	0.98
Bream	19.29	10	70	0.85

Using GHHP's grading scale, grades for each species group were calculated (Table 6), and an overall harbour score and grade determined by averaging the scores of the five species groups.

Table 6. Fish Health Indicator scores and grades for the 2021 Gladstone Harbour Report Card.

	Barred Javelin	Blue Catfish	Sea Mullet	Bream
Standardised HAI score/grade	Grade A Score 0.94	Grade A Score 0.88	Grade A Score 0.98	Grade A Score 0.85
Overall Harbour score	Grade A Score 0.91			

Confidence in scores

As in the 2019-2021 monitoring years, the primary considerations when determining confidence in HAI scores for 2025 are the sample size and the potential for interference by ecological characteristics.

Sample size of blue catfish ($n = 7$) and sea mullet ($n = 11$) were low compared to other species groups, but above the previously determined minimum sample size of 5 (Flint et al., 2018).

The high sample size of barred javelin ($n = 29$) and bream ($n = 42$) should result in lower variability of their measures and therefore provide greater confidence in the results for these taxa.

DISCUSSION

For 2025, the overall Fish Health Indicator grade for Gladstone Harbour, and the grades for each individual species group, were very good (A). This result largely aligns with previous years, when overall grades were A (2021) and B (2020 and 2019).

Field methods

Laboratory and data analysis methods remain unchanged since the indicator was developed by CQUniversity in 2018/19 (Flint et al., 2019). However, the amount of sampling effort has varied between years. These changes to the field sampling regime influenced both the species and size ranges of fish that were caught. For the 2019 Report Card, fish were sampled across two sampling events in Spring 2018 and Autumn 2019 (8 days each). For the 2020 Report Card, fish were sampled only in a single event in October 2019 (7 days). The results for the 2021 Report Card were calculated using data collected across two shorter sampling events (4 days each), in November 2020 and May 2021 and supplemented with data from yellowfin bream collected by hook and line for another CQU research project (Jones et al., 2021). In 2025 the fishing method changed from primarily gillnetting, to angling with baited hook and line over two sampling events in summer and autumn (5 days each), supplemented

by limited netting for mullet (see also Appendix 2). Because the HAI metric analyses the health of each fish individually, the results do not rely on collection being standardised to any one particular fish sampling method.

Both netting and line fishing methods have established roles in fisheries research and monitoring, each with advantages relevant to fish health assessment. Gill nets are a passive gear type that capture fish regardless of behaviour, providing a broad sample of the population. However, they can cause physical damage to fish, which may affect assessments of external condition or tissue integrity.

Hook and line sampling offers a complementary approach by targeting actively feeding fish, often resulting in live captures with minimal injury. This method is advantageous when sample quality is important for physiological or histological analysis (Cooke et al. 2005; Halliday et al. 2011) and for live release of bycatch. While angling can be influenced by behavioural traits such as boldness or feeding motivation (Lennox et al. 2017; Redpath et al. 2010; Biro and Post 2008), it remains a valuable tool for obtaining high-quality fish samples in good condition. Recent studies in tropical estuaries support its use for ecological health monitoring when selectivity is understood and accounted for (Robertson et al. 2021).

To ensure consistency and robustness in long-term monitoring, future sampling should consider combining both gear types or conducting gear comparison studies. This approach would help account for any selectivity biases and improve confidence in trends detected over time. Overall, using both gill nets and hook and line fishing allows for a more comprehensive assessment of fish health in Gladstone Harbour as the preferred taxa can be targeted.

Of the five original fish taxa targeted for the Fish Health Indicator (Flint et al., 2018-2021), barred javelin, blue catfish and bream are those that can most reliably be caught by hook and line. Barramundi (*Lates calcarifer*) can also be targeted using this method, but in much smaller numbers. Due to their high mobility and likelihood of movement between estuaries and sometimes over much longer distances (Flint et al., 2018), the HAI results for barramundi may not always reflect conditions in Gladstone Harbour. For this reason, and because catch rates were low from 2018-21, we no longer recommend targeting barramundi routinely and did not do so in 2025. However, the baseline data collected from 2018-21 could be useful in future if a fish health event occurs in Gladstone Harbour.

Mullet (sea mullet *Mugil cephalus*, and diamondscale mullet *Ellochelon vaigiensis*) were also targeted from 2018-21, but as detritivores they are not readily caught by hook and line, providing another reason to incorporate some gillnetting into the field sampling. In 2024/25, a limitation of angling was the size of fish captured. For barred javelin and blue catfish in particular, the average size in 2024/25 was smaller than in previous years and some of the fish sampled were not yet reproductively mature.

Gladstone Harbour sampling sites

Over the 10 days of sampling, fieldwork was split between the northern, middle and southern areas of Gladstone Harbour, to maintain approximately equal catches between the regions. The fish movement analysis conducted in 2018 detected high transience of fish between different areas within Gladstone Harbour (Flint et al., 2018). The ISP hence elected to report scores of fish health at the harbour-wide scale instead of by zone. Because fish health scores are reported on a harbour-wide scale, the ISP also decided in 2019 to amend the sampling design to allow for higher catches with lower levels of effort (i.e. spend time fishing in zones with a high probability of target species catch, instead of in all zones). This approach was continued when sampling recommenced in 2025 and is recommended for future years.

Reference sites

In 2018, two reference sites were monitored to assist with the development of baselines for fish health measures, Stanage Bay and Baffle Creek. In 2019, the Stanage Bay site was removed. Following that year, only Gladstone Harbour sites have been sampled. Fish from the reference sites did not appear to be in pristine condition, which may reflect local environmental effects. Regardless, in order to continue to assess the condition of fish in Gladstone Harbour in a relative way, it would be beneficial for GHHP to recommence sampling at two control sites (at least) beyond the influence of the harbour, as a precaution against misinterpreting more widespread changes as localised impacts. This was not funded in 2025 but is recommended for the next intensive assessment year.

Health Assessment Index – benchmark and worst-case scenario (WCS)

Since 2018, the benchmark value for calculating report card scores has been 10, and WCS has been 70. These figures were proposed during indicator development, using best available information for different fish species and regions. An individual fish score of 10 means there is minor damage to one organ/metric. For example, a fish with a score of 10 may have a small number of parasites but be otherwise in very good condition, or it may have minor skin abrasions with no other signs of ill health. A score of 70 for the HAI (equivalent to the current WCS) represents damage to at least two organs/metrics. The maximum (worst possible) score for the HAI is 270, an outcome which is unlikely as it all scored organs would show serious, visible

damage and fish in this condition may not survive or may not be well enough to be captured. Considering grading is based on the mean of scores for each taxon, we consider the current benchmark and WCS remain suitable for retention at this time. However, if information becomes available in future to refine the WCS to be more species and region-specific, this should be considered.

The current monitoring framework for Gladstone focuses on individual fish health indicators, primarily using the HAI's gross pathological evaluation method. For the purposes of this report, individual fish health is defined as the structural integrity and physiological function of organs and tissues, including visible lesions, deformities, and parasitic burden (Whitfield and Elliott, 2002). While the HAI approach captures early warning signs of environmental degradation, it can be complemented by broader population and community-level indicators if spatial coverage and sampling intensity allow.

Alternative fish health indicators

In 2018, a range of alternative fish health indicator options were investigated. The main alternatives identified for future investigation were:

- Histopathology
- Bioaccumulation
- Fluctuating asymmetry.

Since then, research at CQUniversity has shed more light on the utility of each of these options in the context of the Gladstone Harbour Report Card.

Histopathology: Recent research has confirmed that histopathological assessment of fish organs as a useful tool for identifying environmental impacts (e.g., da Silva Montes et al., 2020; Assis de Brito Carvalho et al., 2022; Orso et al., 2024). A histopathological assessment of organs collected in the pilot year (2018/19) was conducted by Dr Roger Chong (Flint et al., 2018). This early study could potentially form the basis for a small pilot or student research project to develop a histopathology monitoring and scoring program for the Gladstone Harbour Report Card using the tissues that were collected and preserved in 2025, but the main obstacle will likely remain the ongoing cost of analysis, which is higher than for the HAI. It is possible that this could be managed by selecting fewer organs for analysis and by seeking alternative funding sources.

Bioaccumulation: The 2018 fish health research project (Flint et al., 2018) discussed bioaccumulation of toxicants in fish tissues as a potentially useful indicator for future consideration. Bioaccumulation only becomes an indicator of fish health at levels that cause the initiation of detoxification mechanisms and tissue damage (Whitfield & Elliott, 2002). However, it also provides information on the bioavailability of toxicants in the environment and is an important consideration for fish that are consumed by people. Bioaccumulation is regarded as an integrative measure and an indicator of exposure of organisms to toxicants in polluted ecosystems. Metals are not metabolised by organisms, and therefore, bioaccumulation of metals and metalloids is of particular value (Luoma & Rainbow, 2005). Analyses conducted by CQU found that at the lower levels of exposure generally occurring in Gladstone Harbour, bioaccumulation may not be a universally useful biomarker, although for some combinations of sites, species, tissues and metal(loid)s, further research would be beneficial (Flint et al., 2021b; Jones et al., 2021). Similar to histopathology, the main impediment to implementation of a bioaccumulation monitoring program is likely to be the additional cost associated with laboratory analysis. In 2025, tissue samples have been collected from all dissected fish and stored frozen, to allow future testing for bioaccumulated metals and other toxicants if funding becomes available.

Fluctuating asymmetry: Another alternative biomarker that was flagged for further research in 2018 was fluctuating asymmetry of eye diameter. At the time, the lack of information on 'normal' levels of asymmetry in Australian inshore fishes ruled out this potential indicator. A CQU Masters student, Tania Skewis, studied fluctuating asymmetry in a range of symmetrical traits using fish caught in Gladstone Harbour and surrounds. Fluctuating asymmetry refers to the minute, random deviations from perfect symmetry in morphological characters; it has been used internationally as an indicator of fish health in response to environmental pressures that cause developmental instability (Skewis, 2024). Tania's research found that there was no difference in fluctuating asymmetry between fish from Gladstone Harbour and other sites, and "While fluctuating asymmetry is an interesting potential epigenetic measure of stress, it does not appear to respond to low-level differences in estuary condition and may be better suited to monitoring the effects of specific toxicants or of highly polluted waters." (Skewis, 2024).

Recommendations

Based on the results from the 2024/25 sampling year, and new research that conducted since the last GHHP fish health monitoring in 2021, we propose the following recommendations for GHHP's consideration:

Recommendation 1: Continue to monitor HAI of fish in Gladstone Harbour, with no alterations to lab methods or scoring.

Recommendation 2: For intensive assessment years, use a combination of hook and line for barred javelin, bream and catfish, and gill netting for mullet, over at least 10 fishing days.

Recommendation 3: For interim assessment years, use hook and line to target barred javelin, bream and catfish, over at least 5 fishing days post-wet season.

Recommendation 4: Continue to conduct regionally stratified fish sampling across Gladstone Harbour, but to score and grade the indicator on a harbour-wide scale.

Recommendation 5: If additional budget is available, undertake 2+ days of fishing effort in nearby control sites between the Capricorn Coast and Bundaberg.

Recommendation 6: In the case of a fish health event, target the five taxa for which baseline data are available (barramundi, bream, mullet, barred javelin and blue catfish), in addition to any other teleost (bony fish) species that are showing signs of ill health. Methods could include both hook and line fishing and netting. If a fish health event occurs, Queensland DPI must be notified in advance to confirm approval of additional sampling.

Recommendation 7: Other fish health indicators including histopathology and bioaccumulation of metal(loid)s and other toxicants should be considered for future research and development, should funding become available.

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APPENDIX 1: STATISTICAL ANALYSIS RESULTS

PERMANOVA results for spatio-temporal comparison of HAI scores across sampling seasons (wet vs dry) and zone (north vs central vs south), for bream and for barred javelin.

Source of variation	df	MS	F	P
Bream				
Season	1	885.64	1.8323	0.1877
Zone	2	419.91	0.8688	0.4300
Season x Zone	2	1077.80	2.2299	0.1189
Residual	36	483.35		
Barred Javelin				
Season	1	0.99	0.0040	0.9570
Zone	2	391.59	1.5758	0.2340
Season x Zone	1	139.00	0.5594	0.4520
Residual	24	248.50		

APPENDIX 2: WORKPLAN PROVIDED TO GHHP

Workplan for Project ISP023A 2024-25: Fish Health Indicators for the 2025 Gladstone Harbour Report Card April, 2025

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Background

Fish are key biological indicators of environmental contamination, as they are continuously exposed, ubiquitous in aquatic ecosystems, and play an important ecological role. In 2018, CQUniversity worked with the Gladstone Healthy Harbour Partnership (GHHP) and the Fisheries Research and Development Corporation (FRDC) to develop and monitor fish health indicators for the Gladstone Harbour Report Card. Since this time, indicators such as external features, morphometry, gross pathology, parasite counts and application of the health assessment index (HAI) have been used by the research team to assess a selection of fish species common to the Gladstone region (Flint et al. 2019a, 2020, 2021). The Gladstone Harbour Report Cards of 2019, 2020, and 2021 subsequently incorporated these fish health scores to provide a more comprehensive assessment of the condition of Gladstone Harbour.

In 2024, GHHP commissioned CQUniversity to re-establish the Fish Health monitoring program, starting with a more detailed assessment in 2024–25, which GHHP anticipates repeating every third year. GHHP proposes that a less intensive assessment will be conducted during the intervening years. The indicative budget for the program is ~\$65,000 in the first intensive sampling year, and \$25,000–\$35,000 for each intermediary year.

In the 2024/25 proposal document, the CQUniversity team suggested amendments from previous years that aim to increase both efficiency and the sample sizes per species/taxa. In contrast to previous years when gill netting was used to catch fish, in 2024-25

hook and line fishing was proposed as the primary catch method for target species. A reduction in the diversity of target species was also proposed, as a means of increasing the sample numbers per caught species with the same amount of fishing effort. The fish taxa monitored in earlier years included: barramundi (*Lates calcarifer*), bream (*Acanthopagrus australis* and *A. pacificus*), large mullet (*Mugil cephalus* and *Ellochelon vaigiensis*), blue catfish (*Neoarius graffei*), and barred javelin (*Pomadasys kaakan*).

This workplan has been developed for the consideration of the GHHP Independent Science Panel, following the first sampling event in early 2025.

Field methods and preliminary results

The CQUniversity field team is led by A/Prof Andrew Irving and sampling is conducted in accordance with the conditions of CQU Animal Ethics Committee approval and a General Fishing Permit issued by Queensland Department of Primary Industries. Rise Environmental & Guiding Services (owned by Nathan Johnston) was subcontracted in 2024/25 to provide a suitable vessel and skipper for field sampling in Gladstone Harbour, using hook and line fishing. The first round of sampling for 2024/25 was postponed from the planned dates in December 2024, due to a series of contracting and administrative delays, followed by inclement weather. Sampling was hence conducted over five days from late January through to early March 2025.

Over the five days of field sampling in Jan-Mar 2025, a total of 46 fish were collected and analysed, including:

- Barred javelin (*Pomadasys kaakan*), n = 20
- Blue catfish (*Neoarius graffei*), n = 7
- Yellowfin bream (*Acanthopagrus australis*), n = 19.

Fieldwork was split between the northern, middle and southern areas of Gladstone Harbour, to maintain an approximately similar catch size between the three regions. Bream and javelin were the primary targets, and blue catfish were also retained for analysis when they were captured.

Of the five original fish taxa targeted for the Fish Health Index, the three species listed above (javelin, catfish and bream) are those that can most reliably be caught by hook and line. Barramundi (*Lates calcarifer*) can also be targeted using this method, but in much smaller numbers. Due to their high mobility and likelihood of movement between estuaries and sometimes over much longer distances, the Health Assessment Index (HAI) results for barramundi may not always reflect conditions in Gladstone Harbour. For this reason, we don't recommend continuing to target barramundi routinely. However, the existence of baseline samples from 2018-21 could be useful in future if a fish health event occurs in Gladstone Harbour.

Mullet (sea mullet *Mugil cephalus*, and diamondscales mullet *Ellochelon vaigiensis*) were also targeted from 2019-21, but as detritivores they are not readily caught by hook and line. If GHHP wishes to retain mullet in the report card, this can be achieved by incorporating some gillnetting into the field sampling in the second sampling event for 2025, currently scheduled for five days in May. However, it should be noted that the time taken to gill net will detract from time spent targeting bream, catfish and javelin by hook and line. A further limitation of angling is the size of fish captured. For barred javelin and blue catfish in particular, the average size in 2024/25 was smaller than in previous years. Some of the fish sampled were not yet reproductively mature.

Recommended field methods for intensive assessment years:

- Use a combination of hook and line (for javelin, bream and catfish), and netting (for mullet). However, note the limitations of angling, described above.
- Undertake at least 10 days of fishing effort per year, aiming to catch approximately similar numbers of fish in the three regions of Gladstone Harbour. Effort should preferably be split evenly between pre-wet season (Oct/Nov) and post-wet season (April/May).
- If additional budget is available in an intensive assessment year, undertake an additional 2+ days of fishing effort in nearby control sites in close proximity to Gladstone (i.e., between the Capricorn Coast and Bundaberg). *Note: this was not included in the approved budget for 2024/25.*
- The General Fishing Permit for the project allows for a maximum of 50 specimens of each of the above species to be retained per calendar year.

Recommended field methods for interim assessment years:

- Use hook and line to target javelin, bream and catfish.
- Undertake at least 5 days of fishing effort per year, aiming to catch approximately similar numbers of fish in the three regions of Gladstone Harbour. Timing of the five days to be based on a review of the intensive years' data, likely focusing on the post-wet season in case of any impacts of runoff or flooding.

Recommended field methods in the case of a fish health event:

- In that situation, we recommend targeting the five taxa for which baseline data are available (barramundi, bream, mullet, barred javelin and blue catfish), in addition to any other teleost (bony fish) species that are showing signs of ill health. Methods could include both hook and line fishing and netting. *Note: the approved budget does not include additional sampling in the case of a fish health event.*
- At least two control sites should also be sampled for the same fish species, to provide a comparison to Gladstone Harbour.
- The ethics approval and General Fishing Permit for this project allow for the collection of an additional **150 specimens of 'other teleost species excluding no take species' per fish health event, for diagnostic purposes.** If fish health event sampling is required, DPI must be notified in advance by the field team to confirm their approval of the additional sampling.

Lab methods and scoring

The laboratory method for dissecting fish and scoring the multi-metric HAI was the same as in previous years. Also as in previous years, the following organs were removed during fish dissections: gills, livers, skin/muscle blocks, kidneys, heart, spleen and gonads. 1cm blocks were fixed in 4% formaldehyde for future histopathological analysis (if required), until seven years from the date of collection. Following excision of tissues for fixation, additional samples of muscle, liver, gills and gonad were collected from each fish for frozen storage. While not required by GHHP, these frozen tissues were retained under the principle of gathering as much data as possible from an animal that has been euthanased for scientific research, and are available for other analyses (e.g., metal(loid) concentrations) if funding becomes available.

Recommended lab methods for all assessment years:

- We recommend that GHHP continues to use the same indicator, lab methods, and scoring system as in previous years, with no alterations.